REMARKS

Reconsideration and withdrawal of the rejections of and/or objections to the application are respectfully requested in view of the amendments, remarks and Declaration and attachments thereto herewith

Pursuant to 37 C.F.R. § 1.136(a), Applicants hereby request a two month extension of the term for reply set by the September 13, 2001 Office Action, i.e., up to and including February 13, 2002. Enclosed herewith is a check for $512.00 for a two month extension of time ($200.00) to respond to an official action for a Small entity as well as the fee for four additional independent claims ($168) and sixteen additional claims in excess of twenty for a small entity ($144). The Commissioner is authorized to charge any additional fees to Deposit Account No. 50-0320.

Claims 7, 10, 11-14 and 16-18, 20-22, 24-26 and 28-48 are pending. Claims 9, 15, 19, 23 and 27 are cancelled without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents. Claims 7, 10, 12, 16, 18, 20-22 and 24-26 are amended and new claims 28-48 are added without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

No new matter is added.

It is submitted that the claims, as originally presented, and as herewith presented, are patentably distinct over the prior art cited by the Examiner, and that these claims are in full compliance with the requirements of 35 U.S.C. §112. Changes to claims and/or new claims as presented herein are not made for the purpose of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112. Rather, these changes are made simply for clarification and to round out the scope of protection to which Applicants are entitled.

With regard to the Examiners assertion that the application fails to comply with the requirements of 37 C.F.R. §§1.821-1.825 because the specification allegedly lists nucleic acid sequences which do not have SEQ ID NO identifiers. Applicants have amended the specification to insert SEQ ID NO.s 11, 12 and 13 rendering this objection moot.

Claims 7, 9-21 and 22-27 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite.

Claim 7 is amended and claim 9 is cancelled without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, thereby obviating the rejection.

Claims 12 is amended to delete the recitation "where appropriate" and claim 23 is cancelled without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, rendering the rejection moot.

Claims 13 and 14 are cancelled without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, thereby obviating the rejection.

Claim 18 is amended to recite "active process steps" relating back to the preamble, thereby rendering the rejection moot.

Claim 19 is cancelled without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents thereby obviating the rejection.

Claims 7 and 9-27 are rejected under 35 U. S. C. §103(a) as being unpatentable over Holmes et al. (WO 95/00664). The rejection is traversed.

The Examiner alleges that Holmes teaches an invention which provides nucleic acid molecules for the detection and identification of *Salmonella* species, and for detecting one or more *Salmonella* serotypes and to kits comprising these nucleic acid molecules. The Examiner further alleges that Holmes teaches that 8 oligonucleotide sequences were selected from the sequence and tested for their ability to discriminate between *Salmonella* and non *Salmonella* bacteria and teaches various results in the primer pairs ability to identify and distinguish *Salmonella* from non *Salmonella* bacteria and from different serotypes of *Salmonella.* Holmes specifically teaches evaluation of a Salmonella specific assay and the detection of *enterica, salamae, arizonae, diarizonae, houtenai, bongori* and *indica* and teaches its application in the detection of Salmonella in pork and beef. It is further alleged by the Examiner that the sequences of SEQ ID NOS 1, 3, 6 and 9 are found in SEQ ID NO 1 as taught by Holmes. The Examiner acknowledges that Holmes does not teach the exact nucleic acid molecules "consisting" of the SEQ ID NOs taught in claim 8 but the Examiner alleges that Holmes does allegedly provide the motivation to construct the sequences of claim 8 and the sequences

encompassed by the broadly claimed invention and that therefore the ordinary artisan would have been motivated to construct such nucleic acid molecules as Holmes teaches a need for the detection and differentiation of *Salmonella* for the purpose of controlling infection caused by *Salmonella*.

The Examiner concluded that as the strains and subspecies of the bacteria were known and available in the art at the time of filing, therefore it would have been allegedly obvious to one of ordinary skill in the art to align the sequences of different subspecies of Salmonella for the purpose of providing nucleic acids for detecting and differentiating different subspecies of *Salmonella.* However the Examiner acknowledges that Holmes does not teach the specific sequences consisting of SEQ ID NOS 1, 3, 6 and 9, but allegedly teaches how to align the region of different serotypes of Salmonella to determine regions of similarity and differences in a method of detection. The cited documents do not render the instant invention obvious.

Applicant's invention, e.g., as in claim 7, is directed to, *inter alia*, a set of isolated nucleic acid molecules at least 10 contiguous nucleotides from a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and complements thereof for the detection of all representatives *of Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica* by means of nucleic acid hybridisation or amplification. Such a specific combination of SEQ ID NOs 1-10 or SEQ ID Nos. 1-2 and 6-10 or SEQ ID Nos. 3-10 are not disclosed, taught or suggested by Holmes et al.

It is additionally respectfully asserted that it is well settled that there must be some prior art teaching which would have provided the necessary incentive or motivation for modifying the reference teachings. *In re Laskowski*, 12 U.S.P.Q. 2d 1397, 1399 (Fed. Cir. 1989); *In re Obukowitz*, 27 U.S.P.Q. 2d 1063 (BOPAI 1993). Further, "obvious to try" is not the standard under 35 U.S.C. §103. *In re Fine,* 5 U.S.P.Q. 2d 1596, 1599 (Fed. Cir. 1988). And, as stated by the Court in *In re Fritch*, 23 U.S.P.Q. 2d 1780, 1783-1784 (Fed. Cir. 1992): "The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification." Also, the Examiner is respectfully reminded that for the Section 103 rejection to be proper, **both the suggestion of the**

claimed invention and the expectation of success must be founded in the prior art, and not Applicants' disclosure. *In re Dow*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Holmes does not satisfy the requirements for obviousness. Holmes does not possess the requisite suggestion or disclosure that would lead a skilled artisan to practice, *inter alia*, the instantly claimed set of isolated nucleic acid molecules nucleotides from a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 (claim 7) or SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 (claim 28) or SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 (claim 32) and complements thereof. Furthermore, as shown in Table 2 of Holmes et al. (see page 17), 144 of 146 Salmonella strains (116 of 118 serovars) were correctly identified, while two strains belonging to subspecies IIIa were false negative. Further when PCR was compared to standard culture techniques for the detection of Salmonella in minced meat, a total of 7 of the PCR results were characterized as false positive (see page 27). These results illustrate the unpredictability with regard to the correct selection of specific primer set combinations that will allow the specific and reliable identification of all Salmonella subspecies and also emphasizes the limitations of the primer set used by Holmes et al. Consequently, a skilled artisan would not be motivated to utilize the specific primer sets of Holmes et al. because they do not provide 100% specificity, unlike the primer sets of the present invention.

The cited document, in other words, does not lead a skilled artisan to practice the instant invention.

Further, Applicants submit herewith the Declaration (attached at Exhibit A) of Dr. Kornelia Berghof which clearly demonstrates that the specific combination of SEQ ID NOs. 1-2 and 6-10 allows the specific detection of all 560 strains of Salmonella tested. More specifically, Table 1 demonstrates that the set of isolated nucleic acid sequences of the instant invention is consistently 100% specific for the detection of all representatives of *Salmonella enterica* subspecies.

With regard to the assertion of the Office Action that it allegedly cannot be determined from the recitation in the specification and the previously submitted

Declaration which primer and probe combinations achieved 100% detection of the claimed serotypes. Applicants respectfully submit that primer combination 1: Sa1/Sa2 and Sa6/Sa7/Sa8/Sa9/Sa10 (see instant specification at page 18, lines 12-21) is equivalent to SEQ ID Nos. 1-2 and 6-10 as disclosed in the instant application at page 17, line 18 to page 18, line 7 and SEQ ID No.s 1-2 and 6-10 in the Declaration submitted herein and Sa 1 to 10 to SEQ ID Nos. 1-10 as submitted in the previous Declaration submitted in our June 14, 2001 Response. Consequently the Declaration submitted herein and the previous Declaration are consistent with the teaching in the specification and should be considered by the Examiner.

Further it is explicitly stated at page 18, line 8-11, that sequence sections, i.e., section I (SEQ ID NO: 1 and 2), section II (SEQ ID NO: 3-5) and section III (SEQ ID NO: 6-10), are Sa 1 to 10 used in various combinations.

Consequently, reconsideration and withdrawal of the Section 103 rejection are believed to be in order and such actions are respectfully requested.

Claims 7, 9-21 are rejected under 35 U.S.C. §101 for being directed towards non-statutory subject matter. This rejection is traversed.

Applicants respectfully submit that the amendment if 7, 10, 12, 16, 18, 20-22 and 24-26 without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents have rendered the instant objection moot.

Consequently, reconsideration and withdrawal of the rejections are believed to be in order and such action is hereby requested.

Claims 7, 9-21 and 22-27 are rejected under Section 112, first paragraph, as containing subject matter allegedly not described in the specification in such a way as to reasonably convey to a skilled artisan that Applicants had possession of the claimed subject matter at the time the application was filed. The rejection is traversed.

Applicants respectfully submit that the amendment of 7, 10, 12, 16-18, 20-22 and 24-26 and the addition of new claims 28-48 renders the rejection moot.

The allegations are without merit as possession clearly existed.

The lead case on the written description requirement is *In re Edwards*, 568 F.2d 1349 (C.C.P.A. 1970). The application of that case by the Federal Circuit is the state of

the law on the issue. According to *Edwards*, the function of the written description requirement is to:

> [E]nsure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter later claimed by him; to comply with the description requirement, it is not necessary that the application describe the claimed invention in *ipsis verbis*; all that is required is that it reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him.

(*Id.* at 1351-52) (emphasis added).

Thus, determining whether the written description requirement is satisfied requires reading the disclosure in light of the knowledge possessed by a skilled artisan. Such knowledge can be established by reference to patents and publications available to the public prior to the filing date of the application.

Applying the law to the instant facts, it is clear possession did exist at the time of filing. The present invention is directed to, *inter alia*, a set of isolated nucleic acid molecules at least 10 contiguous nucleotides from a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and complements thereof for the detection of all representatives *of Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica* by means of nucleic acid hybridisation or amplification. Applicants respectfully submit that a skilled artisan would readily understand the method for detecting the presence or absence of all representatives *of Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica* using SEQ ID NOs; 1-10 or SEQ ID Nos. 1-2 and 6-10 or SEQ ID Nos. 3-10.

Further, a skilled artisan, reading the instant specification, would readily understand that possession existed because this same artisan would know the procedures for the specific detection of *Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica* using SEQ ID NOs; 1-10 or SEQ ID Nos. 1-2 and 6-10 or SEQ ID Nos. 3-10 by means of nucleic acid hybridisation or amplification.

Consequently, reconsideration and withdrawal of the rejection are requested.

Claims 7, 9-17 and 22-26 are rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Holmes et al. and Olsen et al. (U.S. Patent No. 6,004,747).

The Examiner alleges that Holmes et al. and Olsen teaches a sequence which comprises the sequence of SEQ ID NOS 1, 3, 6 and 9.

The rejections will be addressed collectively and are traversed.

It is respectfully pointed out that a two-prong inquiry must be satisfied in order for a Section 102 rejection to stand. First, the prior art reference must contain all of the elements of the claimed invention. *See Lewmar Marine Inc. v. Barient Inc.,* 3 U.S.P.Q.2d 1766 (Fed. Cir. 1987). Second, the prior art must contain an enabling disclosure. *See Chester v. Miller,* 15 U.S.P.Q.2d 1333, 1336 (Fed. Cir. 1990). A reference contains an enabling disclosure if a person of ordinary skill in the art could have combined the description of the invention in the prior art reference with his own knowledge of the art to have placed himself in possession of the invention. *See In re Donohue*, 226, U.S.P.Q. 619, 621 (Fed. Cir. 1985).

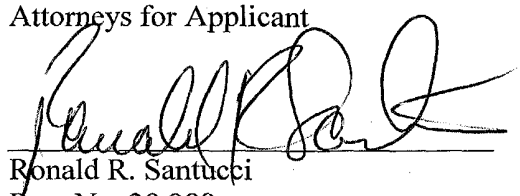
Thus, applying the law to the instant facts, it is clear that Holmes and Olsen do not anticipate Applicants' invention. To wit, Holmes et al. and Olsen do not disclose, suggest or motivate a skilled artisan to, *inter alia*, prepare the specific combination of nucleic acid sequences of the primerset of the present invention. Since Holmes et al. and Olsen do not disclose each and every claimed element of Applicants' invention, Holmes et al. and Olsen are not proper anticipatory references. Therefore, the rejection is obviated.

Consequently, withdrawal of the Section 102(b) rejection is believed to be in order and such action is respectfully requested. Applicants wish to note that included herewith is a change of correspondence address for this application.

In view of the remarks herewith and the Declaration submitted herewith, the present application is in condition for allowance. Early and favorable reconsideration and prompt issuance of a Notice of Allowance are earnestly solicited

Respectfully submitted,

FROMMER LAWRENCE & HAUG LLP
Attorneys for Applicant

By: 

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APPENDIX 1:VERSION TO SHOW CHANGES MADE

IN THE SPECIFICATION

At page 15, line 1 insert:

ST11: AGCCAACATTGCTAAATTGGCGCA (SEQ ID NO:11) (see claim 3, WO 95/00664)

ST15: GGTAGAAATTCCCAGCGGGTACTG (SEQ ID NO:12) (see claim 3, WO 95/00664)

Since, however, no amplification, or only insufficient amplification, occurred with that primer pair with a number of strains of subspecies IIIa, IV, V and VI, in those cases the following primers were used for the PCR and sequencing:

ST11: AGCCAACCATTGCTAAATTGGCGCA (see claim 3, WO 95/00664)

ST14: TTTGCGACTATCAGGTTACCGTGG (SEQ ID NO:13) (see claim 3, WO 95/00664).

At page 19, line 9 insert:

After the end of the PCR reaction, the amplification products were separated by means of agarose gel electrophoresis and visualised by staining with ethidium bromide. The expected product of 167 bp length (primer combination 1) or of 161 bp length (primer combination 2 was observed in all cases in which DNA of strains of the *Salmonella* genus was present (compare Table 1a), but not in the presence of DNA of other tested bacteria (compare Table 1b). After the end of the run, the DNA contained in the gels was transferred by standard methods to nylon filters and hybridised with the oligonucleotide ST14 (TTTGCGACTATCAGGTTACCGTGG (SEQ ID NO:13) (see claim 3, WO 95/00664)) labelled at the 5' end with digoxygenin to test the base specificity especially sensitively. Hybridisation was effected in 5 x SSC, 2% blocking reagent, o.1% lauryl sarcosine, 0.02% SDS and 5 pmol/ml of probe for 4 hours at 60°C. Washing was carried out in 2 x SSC, 0.1% SDS for 2 x 15 minutes at 60°C. Detection was carried out according to standard methods using anti-digoxygenin/alkaline phosphate conjugates in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro-blue tetrazolium chloride (Boehringer Mannheim).

IN THE CLAIMS:

7. (Amended) [A nucleic acid molecule that belongs to a] A set of isolated nucleic acid molecules [by means of which, in a process for the detection of representatives *of Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica*, all the representatives of those subspecies can be detected, or nucleic acid molecule that can be used for such a set,] comprising [wherein, in a region of] at least 10 [successive] contigious nucleotides [of its nucleotide chain, the sequence of the nucleic acid molecule corresponds exactly to a sequence region of at least one representative of the Salmonella enterica subspecies, the sequence region comprising or being a phylogenectically conserved base sequence or a region of that base sequence,] from a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and the complement SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, wherein [in a region of at least] said 10 [successive] contigious nucleotides o[f its nucleotide chain, it is] are 100% or at least 80% identical to [a corresponding number of successive nucleotides of one or more of the following sequences or their complementary sequences:

SEQ ID NO: 1 ATGGATCAGAATACGCCCCG
SEQ ID NO: 2 ATGGATCAGAATACACCCCG
SEQ ID NO: 3 CAGAATACGCCCCGTTCGGC
SEQ ID NO: 4 CAGAATACACCCCGTTCGGC
SEQ ID NO: 5 CAGAATACGCCCCGTTCAGC
SEQ ID NO: 6 CAACCTAACTTCTGCGCCAG
SEQ ID NO: 7 CAACCTAACCTCTGCACCAG
SEQ ID NO: 8 CAACCTAACCTCTGCGCCAG
SEQ ID NO: 9 CAACCTAACTTCTGCGGCAG
SEQ ID NO: 10 CAGCCTAACTTCTGCGCCAG] a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and the complement of SEQ ID NO: 1, SEQ ID NO: 2, SEQ

ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and said nucleic acid sequences allows the detection of all representatives *of Salmonella enterica* subsp. *enterica, salamae, arizonae, diarizonae, houtenae, bongori and indica* by means of nucleic acid hybridisation or amplification.

10. (Amended) The nucleic acid molecule according to claim 7, [which is from] wherein each nucleotide sequence contains 10 to 250 nucleotides [long].

12. (Amended) The set of isolated nucleic acid molecules according to claim 7, wherein each [The] isolated nucleic acid [molecule according to claim 7, which] sequence is present

(i) as DNA, or

(ii) as RNA corresponding to (i), or

(iii)as PNA[, the nucleic acid molecule where appropriate having been modified or labelled in a manner known *per se* for analytical detection processes with a detectable marker].

16. (Amended) The kit according to claim [15] 43, wherein the set of isolated nucleic acid molecules was produced synthetically and [that it was produced] in at least two separate synthesis batches

18. (Amended) A method of detecting the presence or absence of [a] bacteria comprising the [step] steps of :[using a set of one or more nucleic acid molecules according to claim 7 or of a kit according to claim 15 to detect the presence or absence of bacteria belonging to representatives of *Salmonella enterica* subspecies according to claim 7] (i) using a kit according to claim 48; (ii) carrying out nucleic acid hybridisation or nucleic acid amplification or nucleic acid hybridization plus amplification [for detection of] and detecting the presence or absence of all representatives of *Salmonella enterica* subspecies.

20. (Amended) The method according to claim [19] 18, wherein said amplification is carried out by a polymerase chain reaction (PCR) [is carried out as nucleic acid amplification].

21. (Amended) The method according to claim 18, wherein differences between the genomic DNA and/or RNA of the bacteria to be detected and of the bacteria that are not to be detected are determined at at least one nucleotide position in the region of [a] the isolated nucleic acid [molecule according to claim 7] molecules and representatives of a group of bacteria of the *Salmonella* genus are detected.

22. (Amended) The nucleic acid molecule according to claim 10, wherein each nucleic acid sequence contains [which is from] 15 to 30 nucleotides [long].

24. (Amended) The set of isolated nucleic acid molecules according to claim 7, wherein said [The] isolated nucleic acid [molecule according to claim 13, which is a] molecules are modified or labelled nucleic acid molecule in which up to 20% of the nucleotides of at least 10 successive nucleotides of its nucleotide chain are nucleotides that do not occur naturally in bacteria.

25. (Amended) The set of isolated nucleic acid molecules according to claim 14, wherein said [The] isolated nucleic acid [molecule according to claim 14 which is a] molecules are modified or labelled or additionally modified or labelled nucleic acid molecule that comprises, in a manner known *per se* for analytical detection processes, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase, groups for an indirect or direct enzyme reaction.

26. (Amended) The set of isolated nucleic acid molecules according to claim 7, wherein said [The] isolated nucleic acid [molecule according to claim 14 which is a] molecules are modified or labelled or additionally modified or labelled nucleic acid molecule that comprises, in a manner known *per se* for analytical detection processes, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a

solid phase, groups for an indirect or direct reaction using antibodies, antigens, enzymes or substances having an affinity for enzymes or enzyme complexes.

To the United States Patent and Trademark Office

In response to Art unit 1655 of 03/14/01 of Application of

Application NO.: 09/485,434

Filing Date: 04/14/00

Title: Nucleic acid molecule set for detecting Salmonella, nucleic acids, kit and use

Declaration:

Dr. Kornelia Berghof declares:

That she is a citizen of Germany, residing in Berlin, Germany, to which the application identified above has been assigned.

She has carried out the following examples:

Table 1: The following Salmonella strains have been tested positively with the foodproof™ PCR-System of BIOTECON Diagnostics. The detection system was 100% specific in that each strain among the 560 tested was identified as belonging to the genus Salmonella.

Table 1:

Salmonella species	number of isolates tested
Salmonella enterica	
Subspecies enterica (I)	
Serogroup A	1
Serogroup B	120
Serogroup C	127
Serogroup D	79
Serogroup E	38
Serogroup F	9
Serogroup G	8
Serogroup H	5
Serogroup I	4

Salmonella species	number of isolates tested
Serogroup J	1
Serogroup K	1
Serogroup L	3
Serogroup M	10
Serogroup N	3
Serogroup O	5
Serogroup P	4
Serogroup Q	1
Serogroup R	1
Serogroup S	2
Serogroup T	1
Serogroup U	1
Serogroup V	3
Serogroup W	1
Serogroup X	2
	430
Salmonella enterica	
Subspecies salamae (II)	
Serogroup B	2
Serogroup C	2
Serogroup F	2
Serogroup I	2
Serogroup J	2
Serogroup L	2
Serogroup P	2
Serogroup R	2
Serogroup S	2
Serogroup T	6
Serogroup X	5
Serogroup Z	5
Serogroup O : 58	1
	35

Salmonella species	number of isolates tested
Salmonella enterica	
Subspecies arizonae (III a)	
Serogroup J	2
Serogroup K	2
Serogroup P	2
Serogroup R	1
Serogroup S	2
Serogroup U	1
Serogroup V	2
Serogroup Y	4
Serogroup Z	1
Serogroup O : 51	2
Serogroup O : 53	2
Serogroup O : 62	2
Serogroup O : 63	1
	24
Salmonella enterica	
Subspecies diarizonae (III b)	
Serogroup D	2
Serogroup I	2
Serogroup J	2
Serogroup O	2
Serogroup P	2
Serogroup T	2
Serogroup X	4
Serogroup Y	2
Serogroup Z	2
Serogroup O : 53	2
Serogroup O : 60	1
Serogroup O : 61	4
	27

Salmonella species	number of isolates tested
Salmonella enterica	
Subspecies houtenae (IV)	
Serogroup F	2
Serogroup I	4
Serogroup J	2
Serogroup K	2
Serogroup L	2
Serogroup U	3
Serogroup V	3
Serogroup Y	2
Serogroup Z	2
	22
Salmonella enterica	
Subspecies bongori (V)	
Serogroup R	5
Serogroup V	5
Serogroup Y	4
	14
Salmonella enterica	
Subspecies indica (VI)	
Serogroup S	2
Serogroup W	2
Serogroup Y	4
	8
Sum	560

Table 2 is a summary of table 1. Reference is given to the names of the subspecies of Salmonella enterica and Salmonella bongori. All 560 strains have been tested positive with the BIOTECON Diagnostics foodproof™ PCR-System.

Table 2:

Salmonella species	Number of strains tested
Salmonella enterica Subspecies enterica (I)	**430**
Salmonella enterica Subspecies salamae (II)	**35**
Salmonella enterica Subspecies arizonae (IIIa)	**24**
Salmonella enterica Subspecies diarizonae (IIIb)	**27**
Salmonella enterica Subspecies houtenae (IV)	**22**
Salmonella enterica Subspecies indica (VI)	**8**
Salmonella bongori (V)	**14**
	560

Table 3 gives reference to the serotypes of the strains shown in table 1 and table 2.

Table 3:

Sub-species	Sero-gr.	Salmonella Sero type
S. enterica subsp. *enterica*	A	Kiel
	B	Abortusovis
		Africana
		Agona (12 Isolate)
		Arechavaleta
		Brandenburg (12 Isolate)
		Bredeney
		Chester
		Coeln
		Derby (14 Isolate)
		Duisburg (2 Isolate)
		Heidelberg (2 Isolate)
		I 4, 12: d: -
		I 4, 12:-:-
		I 9, 12: l, v: -
		Indiana
		Kiambu
		Kunduchi
		Paratyphi B (8 Isolate)
		Reading
		Saintpaul O5 - (2 Isolate)
		Sandiego
		Schleisheim
		Schwarzengrund
		Stanley
		Stanleyville
		Typhimurium (50 Isolate)
	C	Augustenborg
		Bareilly
		Braenderup
		Choleraesuis
		Choleraesuis var. Decatur
		Choleraesuis var. Kunzendorf
		Colindale

Sub-species	Sero-gr.	Salmonella Sero type
		Livingstone (12 Isolate)
		Mbandaka
		Mikawasima
		Montevideo (6 Isolate)
		Ohio
		Oranienburg
		Oslo
		Richmond (2 Isolate)
		Rissen
		Singapore
		Tennessee
		Thompson (2 Isolate)
		Virchow (11 Isolate)
		I 6, 7 : - : - (2 Isolate)
		Albany (2 Isolate)
		Altona
		Apeyeme
		Bardo
		Blockley
		Bovismorbificans (12 Isolate)
		Charlottenburg
		Cottbus
		Emek
		Ferruch
		Glostrup
		Goldcoast
		Haardt
		Hadar (12 Isolate)
		Kentucky
		Litchfield
		Manchester
		Manhatten (11 Isolate)
		Molade
		München

Sub-species	Sero-gr.	Salmonella Sero type
		Concord
		Infantis (12 Isolate)
		Isangi
		Lille

Sub-species	Sero-gr.	Salmonella Sero type
		Newport (6 Isolate)
		Takoradi
		I 6, 8 : - : -
		I 8, 20 : - : -

Sub-species	Sero-gr.	Salmonella Sero type
	D	Dublin (6 Isolate)
		Durban
		Enteritidis (44 Isolate)
		Gallinarum
		Gallinarum-Pullorum
		Israel
		Javiana
		Kapemba
		Napoli
		Panama (8 Isolate)
		Pullorum (6 Isolate)
		I 9, 12 : - : -
		Typhi (5 Isolate)
		Plymoth
	E	Amager
		Amsterdam O : -, 15+, 34+
		Anatum (8 Isolate)
		Birmingham
		Butantan
		Falkensee
		Give
		Lexington
		London
		Meleagridis
		Münster (2 Isolate)
		Orion (2 Isolate)
		Sinstorf
		Stockholm
		Uganda (2 Isolate)
		Vejle (2 Isolate)
		Weltevreden
		Westhampton
		Zanzibar
		I 3, 10 : - : 6 (monophasisch)

Sub-species	Sero-gr.	Salmonella Sero type
		I 1, 3, 19, : -: -
	F	Chandans (2 Isolate)
		Kisarawe
		Krefeld
		Liverpool
		Rubislaw
		Solt
		Telashomer
	G	Grumpensis
		Havana
		Idikan
		Kedougou
		Poona
		Putten
		Worthington
		I 13, 23, : -
	H	Caracas
		Charity
		Lindern
		Onderstepoort
		Sundsvall
	I	Gaminara
		Hvittingfoss
		Malstatt
		Saphra
	J	Bonames
	K	Cerro
	L	Minnesota (2 Isolate)
		Ruiru
	M	Cotham
		Guildford
		Ilala
		Loeben
		Mundonobo

Sub-species	Sero-gr.	Salmonella Sero type
		I 10 : - : 1,6
		Abaetuba
		Aberdeen
		Cannstatt
		Llandoff
		Senftenberg (2 Isolate)

Sub-species	Sero-gr.	Salmonella Sero type
		Nima
		Patience
		Pomona
		Taunton
		Wedding
	N	Aqua

Sub-species	Sero-gr.	Salmonella Sero type
		Morningside
		Urbana
	O	Adelaide
		Alachua
		Ealing
		Haga
		Monschaui
	P	Lansing
		Roan (2 Isolate)
		Shettfield
	Q	Kokomelemle
	R	Johannesburg
	S	Waycross (2 Isolate)
	T	Waral
	U	Thetford
	V	Koketime (2 Isolate)
		Lawra
	W	Suelldorf
	X	I 47, z_4, z_{23} : - (monophasisch)
		Mountpleasant
S. enterica subsp. *salamae*	B	II 4, 12 : a : - (2 Isolate)
	C	II 6, 7 : d : 1,7 (2 Isolate)
	F	II 11 : g, m, s, t: z_{39} (2 Isolate)
	I	II 16 : g, m, s, t: - (2 Isolate)
	J	II 17 : c : z_{39}

Sub-species	Sero-gr.	Salmonella Sero type
		II 47 : b : 1,5 (2 Isolate)
		II 47 : b : z_6
	Z	II 50 : b : z_6 (5 Isolate)
	O:58	II 58 : l, z_{13}, z_{28}: z_6
S. enterica subsp. *arizonae*	J	IIIa 17 : z_4, z_{32}: - (2 Isolate)
	K	IIIa 18 : z_4, z_{23}: - (2 Isolate)
	P	IIIa 38 : l, v: - (2 Isolate)
	R	IIIa 40 : z_4, z_{24}: -
	S	IIIa 41: z_4, z_{23}: - (2 Isolate)
	U	IIIa 43 : g, z_{51}: -
	V	IIIa 44 : z_4, z_{32}: -
		IIIa 44 : z_{41}, z_{23}: -
	Y	IIIa 48 : (l): -
		IIIa 48 : g, z_{51}: -
		IIIa 48 : z_{36}: -
		IIIa 48 : z_4, z_{23}: -
	Z	IIIa 50 : z_4, z_{24} : -
	O:51	IIIa 51 : z_4, z_{23} : -
		IIIa 51 : g, z_{51}: -
	O:53	IIIa 53 : z_4, z_{23}, z_{32} : -
		IIIa 53 : z_{29} : -
	O:62	IIIa 62 : z_{36} : - (2 Isolate)
	O:63	IIIa 63 : g, z_{51} : -
S. enterica subsp. *diarizonae*	D	IIIb 1, 9, 12 : y : z_{39} (2 Isolate)
	I	IIIb 16 : k : - (2 Isolate)

Sub-species	Sero-gr.	Salmonella Sero type
		II 17 : b : e, n, x, z_{15}
	L	II 21 : z_{10} : - (2 Isolate)
	P	II 38 : d : 1,5 (2 Isolate)
	R	II 1, 40 : z_{42}: 1,5,7 (2 Isolate)
	S	II 41 : z_{10}, 1, 2 (2 Isolate)
	T	II 42 : r : - (6 Isolate)
	X	II 47 : a : 1,5 (2 Isolate)

Sub-species	Sero-gr.	Salmonella Sero type
	J	IIIb 17 : z_{10}, e, n, x, z_{15} (2 Isolate)
	O	IIIb 35 : k: e, n, z_{15} (2 Isolate)
	P	IIIb 38 : l, v: z_{53}
		IIIb 38 : l, v: z_{54}
	T	IIIb 42 : k : z_{35} (2 Isolate)
	X	IIIb 47 : b : z_6
		IIIb 47 : k : z_{35}

Sub-species	Sero-gr.	Salmonella Sero type
		IIIb 47 : r: z_{53}
		IIIb 47 : - : -
	Y	IIIb 48 : (k) : z_{53} (2 Isolate)
	Z	IIIb 50 : k : z
		IIIb 50 : r : z
	O:53	IIIb 53 : l, k : z (2 Isolate)
	O:60	IIIb 60 : z_{52} : z_{53}
	O:61	IIIb 61 : i : z
		IIIb 61 : l, v : 1,5,7
		IIIb 61 : l, v : 1,5,7: (z_{57})
		IIIb 61 : r : z_{53}
S. enterica subsp. *houtenae*	F	IV 11 : z_4, z_{23}: - (2 Isolate)
	I	IV 16 : z_4, z_{32}: -(3 Isolate)
	I	IV 16 : z_4, z_{32}: - (2 Isolate)
	J	IV 17 : z_{29}: - (2 Isolate)
	K	IV 18 : z_{36}, z_{38}: - (2 Isolate)
	L	IV 21: g, z_{51}: - (3 Isolate)
	U	IV 43 : z_4, z_{23}: - (3 Isolate)
		IV 43 : z_4, z_{32}: -
	V	IV 44 : z_4, z_{32}: - (3 Isolate)
	Y	IV 48 : z_{29}: - (2 Isolate)
	Z	IV 50 : z_4, z_{23}:- (2 Isolate)
S. enterica subsp. *indica*	S	VI 41 : b: 1,7 (2 Isolate)
	W	VI 45 : a: e,n,x, (z_{17}) (2 Isolate)
	Y	VI 48 : z_{10}: 1,5 (2 Isolate)
		VI 48 : z_{41} : -

Sub-species	Sero-gr.	Salmonella Sero type
		VI I, v : z_{67}
S. bongori	R	V 40 : z_{35}: - (4 Isolate)
		V 40 : z_{81}: -
	V	V 44 : d: -
		V 44 : z_{39}: - (4 Isolate)
	Y	V 48 : z_{35}: - (4 Isolate)

The strains listed in tables 1-3 have been tested with the PCR test kit „foodproof™ Salmonella" of BIOTECON Diagnostics. This detection system is based on primers and probes SEQ ID 1, 2, 6-10 of US application 09/485 434.

PCR has been set up with all primer and probes of SEQ ID 1, 2, 6-10 combined in one set, i.e. all oligonucleotides SEQ ID 1, 2, 6-10 have been used together in one reaction mix. SEQ nomenclature used in this declaration is identical with the nomenclature of US application 09/485 434:

SEQ ID 1: ATGGATCAGAATACGCCCCG
SEQ ID 2: ATGGATCAGAATACACCCCG
SEQ ID 6: CAACCTAACTTCTGCGCCAG
SEQ ID 7: CAACCTAACTTCTGCACCAG
SEQ ID 8: CAACCTAACCTCTGCGCCAG
SEQ ID 9: CAACCTAACTTCTGCGGCAG
SEQ ID 10: CAGCCTAACTTCTGCGCCAG

Dr. Kornelia Berghof further declares that all statements made herein of her own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements and the like, so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the US Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Potsdam (Germany), 12/03/01

Berghof-J.

Dr. Kornelia Berghof - Jäger